

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Performance Verification of Autoclaves

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1.0 SCOPE AND APPLICATION:

- 1.1 This protocol describes procedures for verifying the performance of the autoclaves. Small changes in temperature within the autoclave produce a large effect on the time required to achieve the predetermined process parameters. It is therefore critical to ensure that the autoclaves are operating within acceptable limits (see ref. 15.1 and 15.2). This protocol also describes the procedure for verifying that the autoclave KILL cycle used to sterilize biohazardous waste is operating within expected parameters.

2.0 DEFINITIONS:

- 2.1 NIST = National Institute of Standards and Technology
- 2.2 Completed Cycle = a complete autoclave cycle includes the recommended 10 minute wait period (indicated on the LED screen on the autoclave) once the door has been cracked open (not greater than one inch). During this time, the steam is allowed to escape and the contents allowed to cool and become acclimated to the ambient temperature to minimize thermal shock, especially to liquids in glass containers.
- 2.3 Chemical Indicator Strips = STEAM*Plus* Steam Sterilization Integrator strips from SPS Medical are engineered to integrate all 3 critical parameters of sterilization (time, temperature and saturated steam) and are certified to perform equal to a biological indicator plus an added safety factor.
- 2.4 Biological Indicator Ampule = Raven Biological PROSPORE Biological Indicator is a hermetically sealed, type I borosilicate glass ampule. The ampule is filled with a modified Soybean Casein Digest Broth containing bromocresol purple acid indicator. Each ampule also contains a population of *G. stearothermophilus* spores. Growth is evident by either turbidity and/or a color change from a purple to or toward yellow.

3.0 HEALTH AND SAFETY:

- 3.1 Laboratory personnel have been trained on the proper use of the autoclaves. Documentation of training can be found in the training file for each employee.

4.0 CAUTIONS:

- 4.1 Since autoclaves use high temperatures, it is necessary to exercise extreme

caution around the device and its associated plumbing. High-temperature surfaces can be encountered even when the device is not in a sterilizing cycle.

5.0 INTERFERENCES:

- 5.1 The maximum registering thermometers should be reset prior to each use as described in section 10.
- 5.2 Shake the thermometer until the column registers 110°C or lower.
- 5.3 The thermometer should be allowed to cool to ambient temperature before it is read. Hold thermometer in an upright position for reading, and only after it has cooled to ambient temperature, or you will obtain a falsely high reading.

6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 Raven Biological Laboratories ProSpore Biological Indicator Ampules with 10⁶ spores of *Bacillus stearothermophilus* (ATCC #7953)
- 7.2 SPSmedical Chemical Indicator Strips
- 7.3 Incubator with temperature set at 55°C ± 1°C
- 7.4 Autoclave #1 located in room B206, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0105898-25
- 7.5 Autoclave #2 located in room B204, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0108298-11
- 7.6 Autoclave #3 located in room B207, Amsco Century Series Scientific Gravity Sterilizer (16×16×26"), Model SG-116, Serial No. 0121198-15
- 7.7 Autoclave #4 located in room B202, Amsco Century Series Scientific Gravity Sterilizer (20×20×26"), Model SG-120, Serial No. 0103000-16

7.8 Autoclave # 5 located in room D122, Tuttnauer Prevacuum Steam Heated Autoclave with Vertical Sliding Door and Steam Generator (52 x 72 x 51”), Model 5596-EP-1V, Serial No. 2311036

7.9 Certified Maximum Registering Thermometers (scale range 80-135°C)

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Maximum registering thermometers must be calibrated against a NIST traceable thermometer and certified annually (see SOP EQ-02, Thermometers).

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Biological indicator ampules (sealed spore ampules containing spores in liquid culture media) must be stored according to manufacturer’s specifications to insure shelf life. Upon receipt, the biological indicators ampules must be placed in the refrigerator.

10.0 PROCEDURE AND ANALYSIS:

10.1 Summary.

Autoclave performance will be verified per run, monthly for short gravity cycles, monthly for short liquid cycles, and monthly for kill cycles as summarized below.

Per run Verification (see Section 10.2)- with every sterilization cycle, verify the performance by examining the autoclave printout, a maximum registering thermometer, and a chemical indicator strip.

Monthly Verification of Short Gravity Cycles and Short Liquid Cycles (see Section 10.3)- on a monthly basis, short gravity cycles are verified on autoclaves #1, #3, and #4. Per run verification and biological ampules are used.

Monthly Verification of Kill Cycles (see Section 10.4)- on a monthly basis, kill cycles (used to demonstrate that the autoclave cycle used to sterilize biohazardous waste is operating within expected parameters) are verified on autoclaves #2, #3, #4, and #5. Per run verification and biological ampules are used.

Refer to Table 1 for a summary of the performance verification practices.

Table 1. Performance Verification Practices for Autoclaves and the Drying Oven

Unit	Location	Use	Per Run	Quality Control		Kill
				Short	Liquid	
Autoclave #1	B206	Media	√	Gravity	√	
Autoclave #2	B204	Kill	√			√
Autoclave #3	B207	Media, kill	√	√	√	√
Autoclave #4	B202	Media, kill	√	√	√	√
Autoclave #5	D122	Kill	√			√

10.2 Performance Verification of Daily Runs (Per Run Verifications). The following data are collected for every autoclave cycle.

10.2.1 Autoclave Printout. For each run, record the minimum and maximum temperatures achieved during the “sterilize” portion of the cycle as indicated on the autoclave printer readout on the appropriate form (see 16.0).

10.2.2 Maximum Registering Thermometer. A maximum registering thermometer is used for each daily autoclave run. Place the thermometer upright in a container and place the container in the autoclave pan along with the items to be processed.

10.2.2.1 Reset the maximum registering thermometer prior to each use by “shaking” the thermometer as you would a fever thermometer. This will force the mercury through the constriction located above the bulb (see 14.1).

10.2.2.2 Record the results from the thermometer on the appropriate form (see 16.0). The thermometer should be allowed to cool to ambient temperature before it is read (see 5.3).

10.2.3 Chemical Indicator Strip. Place the strip flat on top of the container that holds the maximum registering thermometer.

10.2.3.1 Record the results from the chemical indicator strips on the appropriate form (see 16.0). A passing chemical indicator strip must reach the “steam safe” section indicated at the end of the strip. A failing indicator strip does not reach the “steam safe” section at the end of the indicator strip.

- 10.3 Monthly Performance Verification of Short Gravity and Liquid Cycles. On a monthly basis, performance verification is conducted by running a short gravity cycle and a short liquid cycle in Autoclaves 1, 3, and 4. In addition to recording Per Run Verification* data, biological ampules are used.

* For Autoclave #1, use two Maximum Registering Thermometers, two chemical indicator strips, and two biological indicator ampules. One set of thermometer/strip/ampule is placed in the center of the top shelf, and the other set is placed in the center of the bottom shelf.

10.3.1 Place a biological indicator ampule into a test tube containing an appropriate volume of liquid (~10 mL for 20×150 mm test tubes and ~20 mL for 25×150 mm test tubes). Place the test tube containing the ampule in a test tube rack containing 39 other similarly filled test tubes (each rack holds 40 test tubes).

10.3.2 Place the tube with the biological indicator ampule as close to the center of the rack as possible. Place this rack into an autoclave pan along with a maximum registering thermometer, and a chemical indicator strip. Place the chemical indicator strip in the test tube rack near the tube with the biological indicator ampule.

10.3.3 For Autoclave #1, place two pans, each containing a rack with an ampule, thermometer, and strip, as described in 10.3.2, into the unit, one in the center of the top shelf and one in the center of the bottom shelf.

10.3.4 For Autoclave #3 and #4, place one pan containing an ampule, thermometer, and strip, as described in 10.3.2 in the center of the bottom shelf.

10.3.5 Run a gravity cycle for the minimum time necessary to kill all spores as indicated with the QC documentation that accompanies each lot of biological ampules (typically from 15 to 20 minutes).

10.3.5.1 Immediately upon completion of the cycle, remove the pans from the autoclave.

10.3.5.2 Remove the ampule from the test tube and label (location in autoclave). Incubate the ampule as well as one control ampule that has not been autoclaved at 55-60°C for 48±2 hours and record the results on the appropriate form (see

16.0).

10.3.5.3 Repeat the process for a liquid cycle also set for the minimum time necessary to kill all spores as indicated in 10.3.5.

10.4 Monthly Performance Verification of Kill Cycles. On a monthly basis, performance verification is conducted by running a KILL cycle in Autoclaves 2, 3, 4, and 5. In addition to recording Per Run Verification data, biological ampules are used.

10.4.1 To verify KILL cycles, place a biological indicator ampule in the center of an autoclave bag filled with solid waste. Run a standard kill load (3 hour liquid cycle). After completion of the cycle, recover and label the ampule and incubate for 48±2 hours at 55-60°C along with a control ampule that has not been autoclaved. Record the results on the appropriate form (see 16.0).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink. Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

12.2 The autoclave printouts will be collected annually and stored in room D217.

13.0 QUALITY CONTROL:

13.1 NIST traceable Maximum registering thermometers are calibrated annually. See EQ-02, Calibration of Thermometers.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Refer to Table 2 for a complete discussion of nonconformance and corrective action scenarios.

14.2 If a processed biological indicator ampule fails (i.e., growth after incubation at

55°C for 48±2 hours), another ampule should be processed with the same parameters. If the nonconformance persists, service should be scheduled and the autoclave should not be used.

Table 2

Temperature		Chem Strip	Nonconformance and <i>Corrective Action</i> *
Max	Unit		
P	P	F	Check the location of the Chem Strip [†] . Check that the chem strip for the next cycle passes. If the nonconformance pattern persists, run a cycle and process a biological indicator. Incubate and record the results of the biological indicator (see 14.2). <i>Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods.</i>
F	P	P	If a maximum registering thermometer deviates from the sterilize set temperature by greater than 3°C, the cycle is considered to be in nonconformance. Repeat the cycle to verify the nonconformance. If the nonconformance pattern persists, verify the nonconformance again using a different maximum registering thermometer. If the nonconformance persists, run a cycle and process a biological indicator (see 14.2). Call for service. <i>Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods.</i>
F	P	F	Check the location of the Chem Strip [†] . Repeat the cycle to verify the nonconformance. If the nonconformance pattern persists, verify the nonconformance again using a different maximum registering thermometer. If the nonconformance persists, run a cycle and process a biological indicator (see 14.2). <i>Check sterility of media and use if it passes. Repeat the cycle for glassware/dry goods.</i>
P	F	F	The autoclave alarms have been set to trip when the sterilize temperature falls below the sterilize set point (121°C) by more than 1°C. The under temperature alarms will occur during the sterilize phase of the cycle whenever the chamber drain temperature falls below 120°C. If the alarm occurs, an under temperature message will be printed. If the chamber drain temperature rises above the alarm set point (120°C), the alarm will silence and another message will be printed. The autoclaves are set to resume the sterilize phase once the temperature falls within acceptable limits. Consider the unit temperature to have passed and see the appropriate nonconformance if the Maximum registering thermometer or the Chem strip fail. If the total sterilize phase lasts longer than the set time by more than 15 minutes, the Too Long in Sterilize alarm will sound and the cycle will be aborted. A message will be printed and the LED readout will flash "Component Failure." Call for service. <i>If the autoclave goes into alarm but the cycle resumes and is completed successfully, the media should be checked for sterility and performance and can be used if it passes. Repeat the cycle for glassware/dry goods. If the "Too Long in Sterilize" alarm is tripped, media should be discarded and glassware/dry goods should be autoclaved again.</i>
P	F	P	
F	F	F	
F	F	P	

* Italics indicates corrective action to be taken in regards to media being processed at the time the original nonconformance occurred.

[†] The Chem Strip should be placed in a location that is fully exposed to the steam. It should not be placed within the beaker that holds the Max. registering thermometer. If the load includes racks and tubes of media, the Chem Strip should be placed within the rack between tubes.

P = Pass

F= Fail

15.0 REFERENCES:

- 15.1 Bordner, R.H., Winter, J.A., and Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA 600/8-78-017, Environmental Monitoring & Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.
- 15.2 Eaton, A.D., Clesceri, L.S., Greenberg, A.E. eds. 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. American Public Health Association, American Water Works Association, Water Environment Federation.
- 15.3 Lee, C.-H., Montville, T.J., and Sinskey, A.J., 1979. Comparison of the efficacy of steam sterilization indicators. Appl. Environ. Microbiol. 37(6):113-117.

16.0 FORMS AND DATA SHEETS:

- 16.1 Daily Sterilization Record Log Form
- 16.2 Monthly Sterilization Record Form

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Daily Sterilization Record Log Form

OPP Microbiology Laboratory

[illegible]

- 1 Record the cycle as "G" = gravity, "L" = liquid under Type and the duration of the cycle in minutes under Time.
- 2 Record the maximum and minimum temperature achieved during the sterilize phase of the cycle as indicated by the autoclave printout (Unit).
- 3 Record the corrected value for the maximum registering thermometer (Max.) and the serial number of the thermometer.
- 4 Record the results of the chemical indicator strips as "P" for pass or "F" for fail.
- 5 The Sterilization No. indicates the date as well as the unit location and the run number.

Monthly Sterilization Record Form

OPP Microbiology Laboratory

STERILIZER ID AND INFORMATION														
Chem. Strip Lot No./Exp. Date										Bio. Amp. Lot No./Exp. Date				
Room and Unit ID	Cycle ¹		Biological Ampule Results ² Control Amp.= ____		Temperature (°C)						Chem. Strip Results		Sterilization Run No. ⁵	Init.
					Max. Registering Thermometer ⁴				Unit ³					
	Type	Time	Top	Bottom	Top	Bottom	Serial #		Max.	Min.	Top	Bottom		
							Top	Bottom						
B206 #1	G													
	L													
	Kill ⁶													
B204 #2	Kill ⁶													
B207 #3	G													
	L													
	Kill ⁶													
B202 #4	G													
	L													
	Kill ⁶													
D122	Kill ⁶													

- 1 Record the cycle as "G" = gravity, "L" = liquid under Type and the duration of the cycle in minutes under Time.
- 2 Record the results of the ampules after incubation as growth (+) or no growth (0) for the autoclaved ampule (Growth) and unautoclaved control.
- 3 Record the maximum and minimum temperature achieved during the sterilization phase as indicated by the autoclave printer (Unit)
- 4 Record the corrected value for the maximum registering thermometer and the serial number of the thermometer.
- 5 The Sterilization No. indicates the date as well as the unit location and the run number.
- 6 Record the results for the analysis of kill loads (see 10.3.4.8). If not performed, put an ND, "not done."